Ph.D. Synopsis

on

"EXTRACTION, ISOLATION & IDENTIFICATION OF PHYTOCONSTITUENTS FROM MEDICINAL PLANT FOR IT'S ANTI-THYROID ACTIVITY"

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ABSTRACT:

Endocrine disorders are common in India of which the thyroid disorder represents a major subset. In Indian population, thyroid dysfunction prevalence is rising at an alarming rate. Hypothyroidism and hyperthyroidism constitute the maximum percentage of thyroid diseases in India. Alternative medicinal approaches are gaining popularity in view of their efficacy with minimal side effects. various drugs of plant origin which have proven action on thyroid and its functioning and on the various factors associated with thyroid dysfunction. The aim of the current study was to evaluate property of antithyroid on herbal plants as well as natural products by using previous events. The study was carried out by extreme literature survey that invitro and in vivo study model to be using for research. Phytochemical studies grounded in ethno pharmacology are regarded as an effective strategy for discovering new chemical compounds with potential as drug candidates. With this approach, the current study focuses on the "extraction, isolation and identification of antithyroid phytoconstituent from medicinal plant found in Marathwada region" The study will begin with the selection and authentication of a medicinal plant based on its traditional use and reported pharmacological potential. The plant material will be authenticated by a qualified botanist. The authenticated plant material will then subjected to extraction using a Soxhlet apparatus**,** employing a suitable solvent system to obtain an extract. The extract will subjected to phytochemical qualitative & quantitative evaluation. Subsequently, the extract will be evaluated for its pharmacological activities, including antioxidant, anti-inflammatory and anti-thyroid activity. using standard *in vivo* and *in vitro* models. Based on the results, the most potent extract will selected for fractionation using chromatographic techniques. The resulting fraction/s will further be evaluated for their Antithyroid activity to identify the most active fraction. Finally, the bioactive fraction will subjected to advanced analytical techniques, such as LC-MS/GC-MS, to isolate and identify the phytoconstituent(s) responsible for the observed Antithyroid activity.

Keywords:

Extraction, Isolation, Bioactive Compounds, Medicinal Plant, Phytoconstituents,
LC-MS, GC-MS.

INTRODUCTION:

Thyroid diseases are, arguably, among the commonest endocrine disorders worldwide. India too, is no exception. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases.[1] Thyroid diseases are different from other diseases in terms of their ease of diagnosis, accessibility of medical treatment, and the relative visibility that even a small swelling of the thyroid offers to the treating physician. Early diagnosis and treatment remain the cornerstone of management.

Ayurveda describes thyroid disorders based on doshas. Hypothyroidism has been divided into five classifications based on, whether it is caused by - Vata, Pith, Kapha or urinary tract infections (mostly in females) and finally the "goiter" type, where a pronounced swelling of the gland can be seen around the neck region. The "goiter" type is believed to be the advanced stage of Kapha induced hypothyroidism. Improper digestion of food is considered as an underlying cause for all the dosha induced hypothyroidism. In addition, the Vata induced disorder has a psychological component from mental agitation and mental [2]

THYROID GLAND

The thyroid gland is located at the bottom of the neck, above the trachea and bellow larynx that controls the body metabolism and through the bloodstream the thyroid gland releases the thyroid hormones that was stored. Thyroid hormones are responsible for regulating essential activities, such as breathing, heart rate, nervous response, menstrual cycles, and cholesterol level as a part of the endocrine system, thyroid glands produce hormones that reach each cell through the bloodstream. Thyroid follicles are the main components, and the central cavity of follicles is filled with sticky fluids called colloids. A colloid is a center where the thyroid hormones are produced, and the entire production is dependent on one single content called iodine. The food we eat that contains iodine is the primary source of the thyroid. Production of thyroid hormones is carried out in colloids when iodine atoms attach to a glycoprotein called thyroglobulin. Colloid secrets thyroglobulin through follicle cells, and the colloid enzyme links the iodine to thyroglobulin to produce two intermediate hormones triiodothyronine (T3) and thyroxine (T4) These two hormones must be proper rather than too high or too low. Good hormone level depends on the connection between the hypothalamus and the pituitary glands. The hypothalamus produces Thyrotropin Realising Hormone (TRH), which aids in the generation of Thyroid Stimulating Hormone (TSH) by the pituitary gland and regulates

thyroid hormone production. The TRH indicates that the pituitary glands instruct the thyroid gland to produce both T3 and T4 levels can be increased or decreased by increasing or decreasing TSH levels. When T3 and T4 are less, high TSH is released by the pituitary gland in the blood, and when there is high T3 and T4, pituitary gland releases less TSH. That is how there is slow production of thyroid hormones in thyroid glands. The T3 and T4 hormones spread into every cell in the body. The working of cell metabolism is regulated with these hormones. [3]

HYPOTHYROIDISM

Among the various varieties of hypothyroidism, congenital hypothyroidism is probably the most important, as it is requiring an early diagnosis, which is usually followed by appropriate therapy that can prevent the onset of brain damage. Studies from Mumbai have suggested that congenital hypothyroidism is common in India, the disease occurring in 1 out of 2640 neonates, when compared with the worldwide average value of 1 in 3800 subjects.[2]

In childhood too, hypothyroidism can occur. In a clinic-based study from Mumbai, out of 800 children with thyroid disease, 79% had hypothyroidism. Common causes of hypothyroidism in these children were thyroid dysgenesis, dyshormonogenesis, and thyroiditis. [2]

Among adult people in India, the prevalence of hypothyroidism has been recently studied. In this population-based study done in Cochin on 971 adult subjects, the prevalence of hypothyroidism was 3.9%.[3] The prevalence of subclinical hypothyroidism was also high in this study, the value being 9.4%. In women, the prevalence was higher, at 11.4%, when compared with men, in whom the prevalence was 6.2%. The prevalence of subclinical hypothyroidism increased with age. About 53% of subjects with subclinical hypothyroidism were positive for anti-TPO antibodies. This was a population-based study, which used cluster sampling strategy.[3]

HYPERTHYROIDISM

The prevalence of hyperthyroidism has been studied in several studies. In an epidemiological study from Cochin, subclinical and overt hyperthyroidism were present in 1.6% and 1.3% of subjects participating in a community survey.[3]

GOITER AND IODINE DEFICIENCY

Recent population studies have shown that about 12% of adults have a palpable goitre. [3] Autoimmune thyroid disease is probably commoner than iodine deficiency as a cause of goitre in areas that are now iodine sufficient. However, given that iodine deficiency is a problem in India, the importance of iodine deficiency cannot be underestimated in the Indian context.

The link between endemic gaiter and iodine deficiency has been researched in India by several eminent researchers, and this has led to the publication of several important reports. [4,5] Critical research has resulted in endemic goitre being reported from all over the country and not just from the Himalayan and Sub-Himalayan regions. [1]

AUTOIMMUNE THYROIDITIS IN INDIA

Population studies have suggested that about 16.7% of adult subjects have anti-thyroid peroxidase (TPO) antibodies and about 12.1% have anti-thyroglobulin (TG) antibodies. In this same study of 971 subjects, when subjects with abnormal thyroid function were excluded, the prevalence of anti-TPO and anti-TG antibodies was 9.5% and 8.5%.[3]

THYROID CANCER AND INDIA

The Indian Council of Medical Research established the National Cancer Registry Program, and the NCRP has collected the data of more than 3,00,000 cancer patients between the periods 1984 and 1993. [6] Among these patients, the NCRP noted 5614 cases of thyroid cancer, and this included 3617 females and 2007 males. The six centres involved in the studies were at Mumbai, Delhi, Thiruvananthapuram, Dibrugarh, Chandigarh, and Chennai. Among them, Thiruvananthapuram had the highest relative frequency of cases of thyroid cancer among all cancer cases enrolled in the hospital registry, 1.99% among males and 5.71% among females. The nationwide relative frequency of thyroid cancer among all the cancer cases was 0.1%–0.2%. The age-adjusted incidence rates of thyroid cancer per 100,000 are about 1 for males and 1.8 for females as per the Mumbai Cancer Registry, which covered a population of 9.81 million subjects. The histological types of thyroid cancer were studied in a Hospital Cancer Registry of 1185 “new cases” of thyroid cancer.[7]

THYROID DISORDER IN INDIA

Thyroid disease is the ninth most prevalent ailment, according to a survey performed by the Indian Thyroid Society, when compared to other common diseases such as depression, asthma, heart problems, and insomnia. Women are highly prone to thyroid disease, resulting in Weight gain or loss, weakness, and hormonal imbalances compared to men. The prevalence of hypothyroidism has been studied in Indian adults in Cochin. Nine hundred seventy-one adults were chosen, and the prevalence rate of hypothyroidism was 3.9%, and 9.4% subclinical hypothyroidism and females had a higher prevalence rate of 11.4 percent than men, who had a total prevalence rate of 16.2. The prevalence of subclinical hypothyroidism rose with age, and anti TPO antibodies were found in roughly 53% of subclinical hypothyroidism patients. The study was based on population, and a cluster sampling strategy was used. Thyroid disorder in pregnant women results in placental abnormalities and increases the fetus wellbeing risks. [8]

AYURVEDIC BENEFITS OF MEDICINAL PLANT

Ayurveda is an age-old traditional system of medicine originated from India. It is thought to have at least a history of 3000 years. In this system, the universe is believed to be consisting of five elements- Prithvi (Earth), Jala (water), Agni (Fire), Vayu (Air) and Akasha (Space). They are known as Panchamahabhoothas (five major elements). These five elements also constitute the human body and the three humors (tridoshas). The tridoshas are Vata (related to air), Pitta (related to fire) and Kapha (related to water). According to Ayurveda, for a healthy human body, these tridoshas must be balanced. Any imbalance leads to a state of disease. This system of medicine explains the various means to keep tridoshas balanced in various disease conditions, which constitute the treatment part. [9]

Currently there is a lot of interest in getting an Ayurvedic massage -just a superficial part of Ayurvedic treatment- which has got wide popularity across the world. However, the question is, can Ayurveda be a treatment option against emerging health conditions in this century. Can we find solutions for the wide range of lifestyle diseases that we see around us, in Ayurveda?

A study in India published in 2011 reported that, there were 42 million people suffering from some form of thyroid disorder. For hyperthyroidism anti-thyroid medication and surgery are the options, while for hypothyroidism, lifelong thyroid hormone replacement therapy is the option. These

medications can lead to side effects like change in appetite, hair loss, muscle weakness. The ATA has listed Ayurveda as a choice of Complementary and Alternative Medicine (CAM). However, clinical evidence needs to be shown to establish that effectiveness of Ayurveda as a treatment option for thyroid disorders. In this context, we share how patients with thyroid disorders are classified in Ayurveda, how they were treated in our facility with Ayurvedic medicines and how they responded to the treatment.[5]

SIGNIFICANCE OF STUDY

The herbal approach to thyroid dysfunction is invariably necessary to avoid the various side effects of hormonal therapy. The herbal cure is gaining worldwide acceptance and has emphasized the head of the modern scientific exploration and evaluation of ethnos medicine from plants. There is a need for clinical research of the plant to certify their efficacy in normalizing thyroid dysfunction. These will lead to remarkable discoveries from plant-based ethnos medicine.

Studies on antithyroid properties of medicinal plants can help identify compounds that can be used to treat thyroid conditions, such as hyperthyroidism and thyroid autoimmunity. Many plant constituents, such as alkaloids and polyphenols, can interfere with thyroid hormone production and metabolism. Some studies suggest that these compounds may also have antiproliferative effects on thyroid cancer cells. Some plants isoflavonoids have profound effects on thyroid hormones and on the hypothalamus–pituitary axis. Genistein and daidzein from soy (Glycine max) inhibit thyroperoxidase that catalyses iodination and thyroid hormone biosynthesis.

In view of the present study above is related to screen some medicinal plant extracts for its effectiveness in Thyroid disease along with its Anti-Thyroid effects. So, the present study is aimed for-

"Extraction, Isolation and Identification of Phytoconstituents from medicinal plant for its Anti-thyroid activity"

OBJECTIVES OF RESEARCH

Present study is aimed for **"Extraction, Isolation and Identification of Phytoconstituents from medicinal plant for its Anti-thyroid activity"**

with objectives-

a) Phytochemical investigation & standardization of selected Medicinal Plant/s & their extract/s.
b) Screening for secondary activities like Antioxidant activity of plant extract/s.
c) Isolation & Identification of bioactive phytoconstituent/s from the plant extract/s & fraction/s
d) Pharmacological evaluation of plant extract/s and fraction/s for its anti-thyroid activity.
e) Toxicity study of extract/s to obtain safety data

PLAN OF WORK

1. **Literature review**
2. **Collection, identification and authentication of selected plant material**
3. **Processing of plant material**
4. **Pharmacognostic evaluation of plant material**

   Morphological evaluation, microscopic evaluation and powder characteristics
5. **Standardization of plant material**
   - Extractive Value & Ash content
   - Acid & Water-soluble ash
   - Foreign matter
   - LOD
6. **Extraction of selected plant material**
7. **Phytochemical Screening of selected plant extract/s for**

   a) Phytochemical Qualitative analysis
   - Chemical tests of Phytoconstituents
   - TLC study

   b) Phytochemical Quantitative analysis
   - Total Phenolic Content
   - Total Flavonoid Content
8. **Pharmacological screening of plant extract/s for-**

   a) *In-vitro* Antioxidant study

   b) Safe dose calculation by acute oral toxicity studies and repeated dose toxicity study.

   c) Antithyroid activity.
9. **Interpretation of data and its presentation**

Extensive literature survey reveals numbers of medicinal plants are claimed in traditional system for its effectiveness in Thyroid disease so many plants just evaluated for its activity. Accordingly, for present study the following medicinal plants are shortlisted for further literature survey so as to select potent medicinal plant for study.

LITERATURE SURVEY

Extensive literature survey reveals numbers of medicinal plants are claimed in traditional system for its effectiveness in Thyroid disorder so many plants just evaluated for its activity. Accordingly for present study following medicinal plant & their parts are shortlisted for further literature survey so as to select one or two medicinal plant/s for study.

1. *Boerhavia diffusa*
2. *Momordica charantia*
3. *Zingiber officinale*
4. *Coriandrum sativum linn.*
5. *Carica Papaya*
6. *Brassica oleracea*
7. *Spinacia Oleracea*
8. Any other

Literature Review of Activity:

Sr no.	Plant name	Part of the plant	Pharmacological activities	Conclusion	Reference
01	*Curcuma longa L. (turmeric)*	rhizomes	thyroid cancer	Resveratrol, genistein, curcumin, and other phytochemicals have been shown to reduce cell proliferation, viability, growth in diverse thyroid cancer cell lines.	**Javad Sharifi-Rad, et al. (2020)**
02	*Ficus racemosa*	Bark	Anti-thyroid activity	The Antithyroid activity is evident from the decreased T_3 and T_4 levels. It can be said that *Ficus racemosa* significantly by virtue of the presence of tyrosine, which may be useful for further molecular studies to determine the exact mechanism for its Antithyroid activity.	**Mohammed Azharuddin al (2015)**
03	*Luffa acutangula*	peel	Anti-thyroid activity	It is presumed that the antithyroid and anti-hyperglycemic actions of the test plant extract could be the result of antioxidative properties of these phytochemicals.	**Lata Sunhre , AnandKar ,et al (2020)**
04	*Ficus religiosa*	leaf	Anti-thyroid activity	pro-thyroidal potential of Ficus religiosa leaf extract. We postulate that its main bioactive compounds, BA, CGA, and QA involved in this action may serve as novel thyroid agonists in ameliorating hypothyroidism.	**Anand Kar , Durgesh Mahar , et al (2023)**
05	*Aloe gel*	leaf	Anti-thyroid activity	It appears, about 15 compounds, as evidenced by LC-MS/MS study, mostly phenolics are involved in this anti-thyroid effects of the test compound.	**Sunanda Panda, et al (2020)**

06	*Asaparagus Racemosus*	Root	Anti-thyroid activity	The treatment of aqueous concentrate of the root of Asparagus Racemosus have indicated noteworthy changes in thyroid hormone level and lipid profile level in diverse exploratory gatherings of rats.	**Santhi T, al (2019)**
7	*Piper nigrum*	Fruits	Anti-thyroid activity	A higher dose of piperine may inhibit thyroid function. when Swiss albino mice were orally administered with 2.50 mg/kg piperine for 15 days, it lowered the serum levels of both the thyroid hormones, thyroxin (T4) and triiodothyronine (T3)	**Shain M Pathan, et al (2023)**

Literature Review of Plants:

Sr no.	Plant name	Part of the plant	Pharmacological activities	Conclusion	Reference
01	*Boerhavia diffusa*	Leaves	Anti-inflammatory, antioxidant activity	The Plant ethanolic extract Showed a significant traditional application. In vitro assays are conducted to asses effect of *Boerhavia diffusa or isolated compound on various cellular processes.theseassay can include measuring antioxidant activity*	**Pravin v.borse et al. (2023)**
	Boerhavia diffusa	Roots	Phytochemical evaluation	the powder of roots gives therapeutic potential of the drug Punarnava (Boerhaavia diffusa Linn.) is based on various phytoconstituents such as tannin, phenols, alkaloids, flavonoids, proteins, steroids.	**Divya Raj et al. (2024)**
	Boerhavia diffusa	Leaves	Antioxidant, thrombolytic, cytotoxic, antimicrobial activities	The results showed that the plant extracts were a rich source of phytoconstituents. Methanol extract showed higher antioxidant, thrombolytic activity and less	**Apurba surker apu, mahabuba sultan lizza,ATM**

				cytotoxic activity than those of *n*-hexane and ethyl acetate extracts of *B. diffusa*. Among the bioactivities, antioxidant activity was the most notable compared to the positive control and thus could be a potential rich source of natural antioxidant.	**Jallaluddin et al 2012**
	Boerhavia diffusa	Roots	Urolithiasis Activity (*Mutrakrichra*)	The aqueous extracts of root parts of Punarnava (*B. diffusa*) was prepared and preliminary phytochemical analysis were performed by using the following standard methods. the prepared tablet dosage form of *Punarnava ghan* was effective in the management of urolithiasis.	**Dileep Singh Baghel, Amit Mittal, Saurabh Singh et al 2021**
	Boerhavia diffusa	Leaves	antioxidant and antibacterial activities	The ability of the extract to scavenge free_radicals_against 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay The result ranged from 51.05% in ABD to 78.61% in ELS extracts, respectively. The result showed invariable dependency between plant species/materials and the DPPH scavenging power. the antibacterial activities of the leaf extracts, after testing them against some selected bacteria, is shows The zone of inhibition of the leaf extract against Staphylococcus aureus, *Pseudomonas aeruginosa and Bacillus cere us ranged from 7 to 14 mm and 2–3 mm, respectively*	**Emmanuel Adeku, *et al* (2018)**
02	*Momordica charantia*	Fruits	Analgesic and anti-inflammatory	The Methanolic extract of Momordica charantia (Mc-ME) exerts its anti-inflammatory activity by reducing the action of transforming growth factor	**Yang, Woo Seok, e*t al* (2020)**

				beta-activated kinase 1 (TAK1), which also affects the activation of NF-kappa B and AP-1. This extract blocks NO production in a dose-dependent manner. It also decreases the mRNA expression levels of inducible NO synthase (iNOS) and cyclooxygenase (COX)-2. It also decreases the levels of p65 [a nuclear factor (NF)-kappa B subunit] and c-Fos [an activator protein (AP)-1 subunit] which is indicate by Luciferase assays and nuclear lysate immunoblotting analyses.	
	Momordica charantia	Fruits	Antimicrobial activity	The Momordica charantia L. shows strong antimicrobial potential, with bactericidal and fungicidal profile, there is the prospect to constitute a new therapeutic strategy for the control of infections, particularly in multiresistant strains The ethanolic extract of Momordica charantia L. used as a source of plant-derived natural products with resistance modifying activity . The essential oil obtained from the seeds of Momordica charantia and which is test for its antibacterial and antifungal activities.	**Wang, Shuzhen, *et al* (2016)**
	Momordica charantia	Fruits	Analgesic and anti-inflammatory	The Methanolic extract of Momordica charantia (Mc-ME) exerts its anti-inflammatory activity by reducing the action of transforming growth factor beta-activated kinase 1 (TAK1), which also affects the activation of NF-kappa B and AP-1.	**Yang, Woo Seok, et al.**

	Momordica charantia	Fruits	Hepatoprotective effect	The aqueous extract of Momordica charantia significantly restore the liver weight to near normal in CCl4 induced liver damaged rats. Also, treatment with the extractcause a significant increase in Heamoglobin and Packed Cell Volume, decrease in the activities of Alanine Transaminase (ALT),Aspartae Transaminase (AST), Alkaline Phosphatase (ALP) and level of total bilirubin.	**Pereira. B. S, et al. (2016)**
	Momordica charantia	Fruits	Larvicidal Activity	evaluated the larvicidal, acaricidal and pediculicidal effect of synthesized zinc oxide nanoparticles (ZnO NPs) using Momordica charantia leaf extract against the larvae of Rhipicephalus microplus, adult of Pediculus humanus capitis, and the larvae of Anopheles stephensi, Culex quinquefasciatus.	**Gandhi. P, et al. (2017)**
	Momordica charantia	Fruits	Antidiabetic Activity	The alcoholic extract of karela pulp demonstrates a significant hypoglycemic activity in various experimental models of diabetes. The extract also increases the rate of glycogen synthesis from 14C-glucose by 4-5 folds in the liver of experimental animals.	**Han, Joo-Hui, Nguyen Quoc, Tuan, Park et al. (2018)**
	Momordica charantia	Fruits	Hypolipidemic Activity	studied the hypolipidemic effect of different fractions of methanolic extract of Momordica charantia (Linn.). The different fractions (Petroleum ether, Ethyl acetate and Chloroform) exhibits corrections of altered biochemical parameters and that indicates the plant have	**Hossain. M.S, et al., (2012)**

				favorable effect in hypolipidemic activity.	
3	*Zingiber officinale*	Roots	Anti-bacterial Activity	the antibacterial activity of the extracts of ginger's *(Zingiber officinale)* ethanol,chloroform ,and n-hexane extracts dissolved in DMSO (dimethyl sulfoxide)(5%) and distributed evenly among six wells, (100 µL) in each well. The concentrations were 50 mg/mL, 100 mg/mL, and 200 mg/mL, respectively. Each test was performed three times.The presence of inhibition zones were measured using Vernier caliper recorded and considered as indication for antibacterial activity.	**Great Iruoghene Edo *et al* (2024)**
	Zingiber officinale	Roots	anticancer activity	the ginger root extract prepared by the Soxhlet method (G3) had the strongest activity, and showed significant differences compared to extracts from methods G1 and G5, on the hepatocellular carcinoma cell lines cultured in both 2D and 3D conditions.	**Sinh Truong Nguyen,Phuc Hong Vo, et al(2019)**
	Zingiber officinale	Roots	Anti-Inflammatory and Antioxidative activity	ginger ethanolic extraction via HPLC to have a 6-gingerol concentration. Four groups induced peritoneal adhesion to evaluate ginger's effects on peritoneal adhesion. Then, ginger extract (50, 150, and 450 mg/kg) was administered by gavage in various groups of male Wistar rats (220 ± 20 g, 6–8 weeks). After sacrificing the animals for biological assessment, macroscopic and microscopic parameters were determined via scoring	**Roghayeh Yahyazadeh , et al (2023)**

				systems and immunoassays in the peritoneal lavage fluid.	
	Zingiber officinale	Roots	Anti-Bacterial Activity	The antibacterial activity increased from 25% percent and above with the rise in the concentration of aqueous ginger extract, and selenium nanoparticles had no impact at 0.5 percent, 1 percent, 2 percent concentration.	**Ahmed Ghaith Ahmed, et al (2022)**
	Zingiber officinale	Roots	Antimicrobial property	Cultures of E. Coli, Bacillus subtilis, St. Coccus aureus and St. faecalis to identify the antimicrobial strength. Effectiveness of ginger against different conditions attributed to its different constituents (volatile oils, shogaols, Gingerol, diarylheptanoids) that show their therapeutic efficacy by modulating the genetic or metabolic activities of our body	**Humayun Riaz, et al (2015)**
04	*Coriandrum sativum linn.*	Seeds, Leaves and Stems	Antibacterial Activity	This study showed that hot aqueous extracts for 1hr. to all parts of coriander indicated no antibacterial activity, while cold aqueous extract for 24hrs of coriander seeds had inhibitory effects for some tested bacteria but leaves and stems did not.	**Intisar M. Oudah, et al (2010)**
	Coriandrum sativum linn.	Leaves and Stems	Obesity and Metabolic Syndrome	coriander seeds and leaves have the potential to ameliorate obesity and its associated risk factors due to their powerful antioxidant and anti-inflammatory activities.	**Samir Scandar, et al (2022)**
	Coriandrum sativum linn.	Leaves and Stems	Cardiovascular Activity	The included studies in this systematic review shed light on the cardioprotective ability of coriander; however, more in vitro studies are needed to elucidate the mechanism of action of its extracts in cardiovascular diseases.	**Nisa Najibah Mahleyuddin, et al (2021)**

	Coriandrum sativum linn.	Leaves and Stems	Antidiabetic Activity	The activity of coriander leaves as an antidiabetic agent, utilizing both vivo and in vitro method. In vivo method, the ethanol extract was orally administered to the insulin deficiency mice model (alloxan induced at dose of 65 mg/kg bw iv) with dose of 200, 400, and 800 mg/ kg bw for 14 days. At the end of the in vivo study, the pancreas was isolated. While in vitro method, the α-glucosidase inhibition activity study was performed.	**Widhya Aligita, et al (2018)**
	Coriandrum sativum linn.	Leaves, seed and Stems	Antioxidant activity	seeds and leaves from coriander have concentration-dependent inhibitory activity towards 15-LO and radical-scavenging properties. However, the effects are more potent in extracts from leaves than in seeds from coriander and it seems that compounds of medium polarity are most potent, even if their total antioxidant contribution in the plant is small.	**Helle Wangensteen al (2004)**

MATERIALS AND METHODS

- **Plant Material:**

  The selected plant part/s will be collected from known sources and authenticated by an expert botanist.

- **Processing of Plant Material:**

  The collected crude drugs will be processed according to official standards, including steps such as drying, grinding, and sieving [10].

- **Standardization of Plant Material:**

  Phytochemical examinations will be conducted using standard methods, including the estimation of extractive value, acid and water-soluble ash, foreign matter, and loss on drying (LOD) etc

- **Pharmacognostic Evaluation of plant material:**

  The parts of the selected plant/s will be subjected to Pharmacognostic evaluation, including morphological evaluation, microscopical evaluation, and powder characteristics etc [11]

- **Extraction of Plant material:**

  The powdered form of the selected plant material will undergo extraction, with the choice of solvent and extraction method determined based on the chemical constituents of the selected plant parts and the nature of those constituents. [12].

- **Phytochemical Screening of selected plant extract/s for**

  Qualitative analysis will be conducted to detect phytoconstituents, which will then be confirmed using suitable chromatographic techniques.

  Quantitative analysis will be performed to determine the total polyphenols, total flavonoids etc [1].

- **Pharmacological screening of plant extract/s**

  The extract/s of the selected plant parts, either individually or in combination, will be evaluated for

  i. ***In vitro*** **Antioxidant study**.

  In vitro antioxidant activities for the extract/s will be carried out using appropriate *in-vitro* antioxidant model like; DPPH assay

DPPH Assay: 1, 2-Diphenyl-2-picryl hydrazyl Radical (DPPH)

Initial volume 0.1 mL of various concentrations of samples was mixed with 0.4 mL of 0.3M DPPH reagent prepared in ethanol. The mixture was shaken thoroughly and incubated in the darkness at room temperature for 30 min. The absorbance of the reaction was measured spectrophotometrically at 517nm, immediately after mixing and then after incubation as well. The scavenging effect of DPPH free radical was calculated by using the following equation [14].

% Scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100

Where control is the absorbance of the blank (a reaction with all the reagents except the test extract), and absorbance of sample is the absorbance of the test extract. Tests were carried out in triplicates to obtain 50% inhibition (IC50), using Butylated hydroxy Toluene.

ii. **Safe dose calculation:**

The safe dose calculation of plant extract/s for efficacy evaluation will be conducted in accordance with OECD Guidelines for Acute Toxicity Study: Class Method (423)

Repeated dose oral toxicity study

Repeated dose oral toxicity study of plant extract/s will be carried out as per OECD Guidelines for 'Repeated dose oral toxicity study: Class Method (407)

iii. Antithyroid activity

Thyroid hormones level (T3, T4, TSH), Histopathological model, etc. can be used to study the Antithyroid activity of various plant extracts.

- **Isolation & identification of Antithyroid bioactive constituent**

i. Selection of extract/s

After preliminary phytochemical and pharmacological evaluations for antioxidants, anti-inflammatory, and antithyroid activities, the most potent extract/s will be selected for further studies.

ii. HR-LCMS Study of selected extract:

The selected extract/s will be subjected to HR-LCMS studies to confirm the presence of various phytoconstituents in extract/s.

The abundant bioactive phytoconstituent will be subjected for further study**.**

iii. **Fractionation:**

Further as per the results of HR-LCMS, the extract/s will be subjected to fractionation for purification & isolation of bioactive phytoconstituent & the fractionation will be done by using solvent fractionation (A technique used to separate components of a mixture by exploiting differences in their polarity)

iv. **Phytochemical evaluation of fraction/s:**

The resulting fraction/s will undergo qualitative analysis, including identification tests, and HPTLC studies.

v. **Identification of phytoconstituent/s**

The phytoconstituent/s will be identified by using LC-MS / GC-MS spectroscopic techniques to confirm presence of bioactive constituent in fraction/s.

- **Interpretation of data and its presentation:**

- Data collected that for Pharmacognostic evaluation of selected plant's part will be presented in tabular form and microscopic observation will be presented as figures of TS & for powder characteristics.
- Qualitative evaluation of plant extracts for the content of phytoconstituents will be presented as either present (+ve) or absent (-ve) and presence of phytoconstituents will be confirmed by TLC by comparing with Rf value of standard. Fractioned extracts will be subjected for phytoconstituents characterization by HPTLC.
- Safe dose will be calculated as $1/10^{th}$ dose of LD_{50} value of plant extracts. Repeated dose toxicity study data along with Histopathological findings will be presented.
- All the data related to efficacy of extracts of selected plant's part will be subjected to statistical analysis like student's t test, ANOVA etc. to find level of significance between extracts and between extracts to control/positive control data. Such data will also be presented as chart diagram.

YEAR WISE PLAN OF WORK:

Sr. No.	Work	Period in months
1.	Literature survey	06
2	Plant collection and preliminary screening	03
3	Extraction of plant material	05
4	Phytochemical evaluation	04
5	Pharmacological screening	08
6	Pharmacological evaluation of fractions	04
7	Isolation and Identification of Phytoconstituents/s	02
8	Compilation of data & Thesis writing	04
	Total duration of project	**36 months**

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